Conducting Paper Based Biosensor For Glucose Detection

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We hereby certify that the Project Dissertation titled **"Conducting Paper Based Biosensor For Glucose Detection"** which is submitted by **Geetu P Paul, 2K17/PTE/03,** Department of Applied Chemistry, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the Master of Technology, is a record of the project work carried out by the student under our supervision. To the best of our knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

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ABSTRACT

In this work, efforts have been made to fabricate a paper based sensor comprising of PEDOT: PSS and reduced graphene oxide/ TiO_2 nanocomposite. The effect of different solvent (Glycerine, Ethylene glycol, Dimethylsulfoxide, Methanol) on the conductivity of the paper has also been investigated. The conductivity of the PEDOT: PSS coated paper significantly increases from 6.9 x 10^{-5} S/cm to 1.1 x 10^{-4} S/cm on treatment with Ethylene glycol. Further the modification of conducting paper with synthesized rGO/TiO_2 nanocomposite shows better electrochemical properties. The fabricated conducting paper was utilised for glucose detection shows high sensitivity (83.86 μA M^{-1}). This paper electrode may be a promising substitute over other expensive conventional electrodes.

Keywords: Glucose, PEDOT: PSS, TiO_2 nanoparticle, Graphene oxide, Biosensor

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List of abbreviations

PEDOT: PSS	Poly (3, 4-ethylenedioxythiophene):poly (4-styrenesulphonate)
rGO	Reduced graphene oxide
TiO_2	Titanium dioxide
GOx	Glucose oxidase
GDH	Glucose-1-dehydrogenase
DM	Diabetes mellitus
FAD^+	Flavin adenine dinucleotide
CP	Conducting paper
DW	Distilled water
FTIR	Fourier transform infrared spectroscopy
XRD	X-ray diffraction
TGA	Thermogravimetric analysis
DSC	Differential scanning calorimetry
SEM	Scanning electron microscopy

1. INTRODUCTION

Diabetes Mellitus (DM) is a prominent healthcare concern in most countries, and is the most common endocrine disorder of carbohydrates metabolism worldwide. According to WHO report, nearly 170 million persons (2001) all over the world were reported to have DM, and this number is likely to rise to 366 million people by 2030. It is estimated that the occurrence of DM among adults are 6.4%, and by 2030 it may increase upto 7.7%, thereby affecting 439 million adult people. Several methods are being used for the diagnosis and management of DM[1] but still there is a need for highly sensitive and cheaper detection method for detection of DM.

DM is a metabolic disorder characterised by high blood sugar level (hyperglycemia), which is a condition of having excessive amount of glucose in the blood plasma. Currently, for the detection of glucose levels in the human body, many glucose detecting devices have been reported. Such as infrared spectroscopy [2], fluorescence spectroscopy [3], Raman spectroscopy [4], liquid chromatography [5], polarimetry [6]. However, these instruments are complex and expensive and requires skilled manpower. Thus, it is necessary to develop a simpler instrument for detection of DM by measuring the glucose level in the body. In this context biosensor is a simpler and precise method for glucose .Biosensor is an analytical device, used for the detection of chemical substances that combines a biological component with a physicochemical detector [7].

In recent years conducting paper (CP) has evolved as the ideal platform for the desired analytical and clinical bio-sensing applications. CP can act as a suitable substrate for chemical and biological analysis as it provides large surface to volume ratio that enables the storage and flow of liquid. Further, these CP requires a small quantity of samples and possess a porous structure that facilitates immobilization of sensing materials by suitable surface modification [8].In comparison to other traditional substrates (glass, ceramic, and polymer), paper-based substrate is easy to develop, cheaper, biocompatible, portable, flexible, disposable, and environment-friendly[9]. For making the paper conducting,

various inorganic and organic materials have been widely used. In this context, biosensors are considered to be simpler and conducting polymer and their composites are a promising material for fabrication of CP[10]. Polymers with π electrons are said to be conducting polymers and delocalization of these π electrons with rapid electron transfer and solution processing facility leads to the promising application of conducting polymer to obtain conducting paper for biosensing applications. Further, the doping of conducting polymer with particular dopants (graphene, metal nanoparticles, metal oxide nanoparticles) enhances its physical, optical, electrical, chemical, and electrochemical properties [11].

Among the various conducting polymers, poly (3, 4-ethylenedioxythiophene): polystyrene sulfonate (PEDOT: PSS) has turned a new page for the paper-based biosensing application because of its stability and homogeneous entrapment of the materials on the paper. Moreover, solvent treatment with particular dopants significantly enhance the conductivity of PEDOT: PSS and further enhances its electrochemical performance in terms of sensitivity and stability [12].

Among various detection approaches, electrochemical techniques turned out to be the desired method for paper based qualitative and quantitative analysis. The electrochemical methods offer fast response time, high sensitivity, transferability and high signal to noise ratio [13]. The coupling of conducting paper with proper enzyme provides a proficient platform for conduction of both ionic and electronic charge carriers that play a main role in transduction among required biomolecules [14].

In the present electrochemical biosensor based on conducting paper has been fabricated. Further the incorporation of rGO/TiO_2 into the conducting paper results in improved electrochemical properties. This low cost, flexible CP based platform shows high sensitivity for the detection of glucose.

2. LITERATURE REVIEW

2.1 Biosensor

Biosensor is an analytical device which incorporates a biological sensing element integrated with a physiochemical transducer that measures the sensitivity and specificity of biochemical reaction to deliver complex bioanalytical measurement [15].

It consist of mainly two parts

(i) Bioreceptors: Biomolecule that analyse targeted analyte.

(ii) Transducer: Converts the biochemical signal into measurable signal.

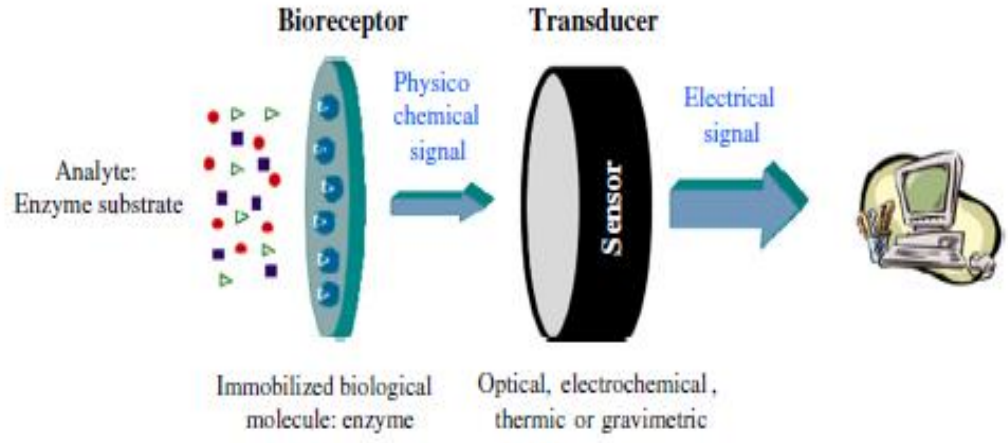

Fig 1.1 Schematic representation of a typical biosensor [15]

The biologically derived parts mainly includes microorganisms, receptors, nucleic acid, enzymes, lectins, antibodies etc [16]. The main transducers are electrochemical, piezoelectric, optical, and magnetic types. Electrochemical biosensors have been widely used in biosensor fabrication as they show excellent selectivity, sensitivity, stability, reproducibility, and quick maintenance with minimum cost. Electrochemical biosensors are of four types ie impedence, amperometric, conductometric, and potentiometric types [17].

2.1.1 Electrochemical biosensor

Electrochemical biosensors have received substantial attention as they are cost-effective, sensitive, and selective. They consumes electrons by enzymatic catalysis reaction (those enzymes are called redox enzymes). The electrochemical biosensors usually consist of working electrode, a reference electrode (AgCl), and a counter electrode (Pt). The reaction takes place by electron transfer across the double layer and the specified analyte is targeted at electrode surface from the sample solution by producing a current or voltage. [18].

2.1.2 Methodology for glucose detection

The basic principle of glucose measurement depends on the interaction with one of the three enzymes ie glucose oxidase (GOx), hexokinase, or glucose-1-dehydrogenase (GDH). Among these, hexokinase is used in spectrophotometry method as a reference for determining glucose content, but only in clinical labs [21]. GOx and GDH are mainly using enzymes for glucose detection. These enzymes varies in turnover rate, redox potentials, and selectivity for detection of glucose. GOx is considered as the standard enzyme for biosensors as it has high selectivity for glucose, cheaper, easy to attain, and can withstand change in pH, ionic strength, and temperature than other enzymes [22].

The immobilised GOx catalyzes oxidation of ß-D glucose by molecular oxygen and results in the production of gluconic acid and hydrogen peroxide (figure 2.1) [23]. Also, to carry out this process, a redox cofactor (flavin adenine dinucleotide (FAD^+), which is an electron acceptor) is required for oxidation. During this redox reaction, FAD^+ is reduced to $FADH_2$. Concentration of glucose can be analysed by the amount of H_2O_2 produced which can be correlated by the transferred amount of electron at anode [23].

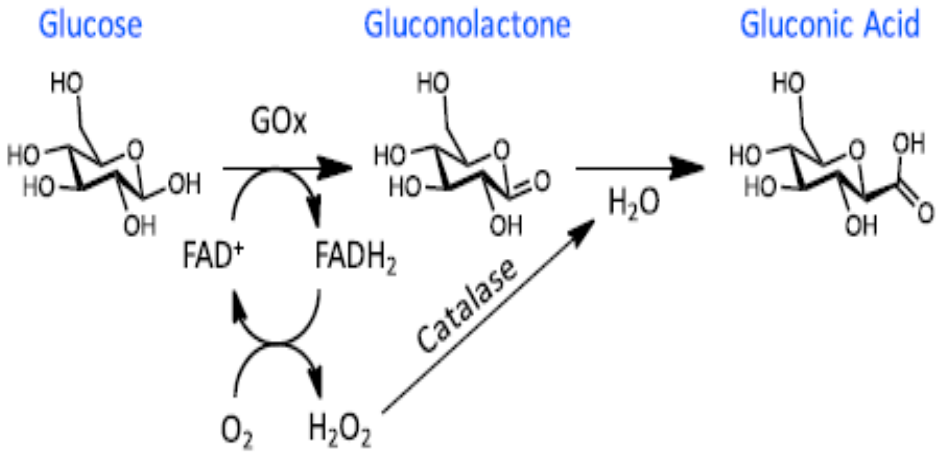

Fig 2.1 Oxidation of glucose to gluconolactone using GOx and then conversion to gluconic acid.[23]

This sensor measures the formation of hydrogen peroxide with decrease in oxygen concentration and thus detects glucose correspondingly [16]. The main obstacle to overcome is the interference of other electroactive species like ascorbic acid, creatinine, and xylose. Figure 2.2 shows the pictorial representation of the working model glucose detecting biosensor. [24].

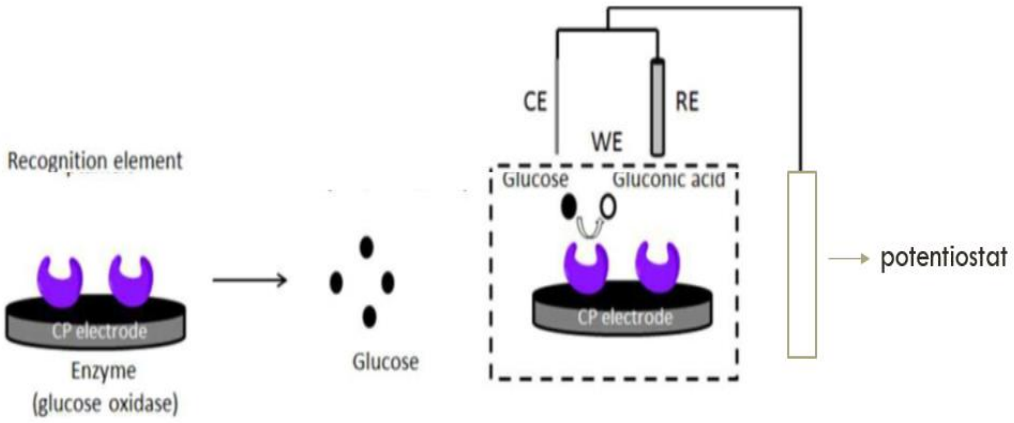

Fig 2.2 Pictorial representation of glucose biosensor [24].

For detecting glucose, electrochemical technique having biological enzymes with nanoparticles have been primarily used, but still, there is a need of non-invasive method, highly flexible and reproducible with resistant to interferential materials

device. Lots of researches based on the glucose detections by invasive methods have been studied and technological advances have been achieved on that, still improvements for more commercialisation are required in areas such as economy, long term stability, and person dependent calibration [25]. When developing a non-invasive method for glucose detection, more stable product for long period of time is convenient with efficient block inferential species in analyte sample and thereafter enhancement in electron transfer is also the most important concern to be noted. Fabrication should be feasible with low cost showing rapid assay time. Recently, a non-invasive glucose meter has been developed and commercialized by using urine and showed stable and measurable results by blocking inferential materials [26]. Conducting nanoparticles have been utilised for the acceleration of electron transfer systematically on the surface of electrodes. Previous studies have mentioned direct attachment of nanoparticles onto graphene oxide for the electrode fabrication, and their corresponding electrochemical characteristics to determine the glucose concentration [27]. It has been well described in latest studies that incorporation of nanoparticles have significantly improved glucose detection by improvement in electron transfer efficiencies when compared those without nanoparticles [28].

2.2 Fabrication of conducting paper

The fabrication of CP for biosensing application can impact the simplicity of sensing. By altering the surface characteristics of cellulose fibre (chemical processing or physical adsorption) we can modify that paper for biosensing application. Some of the methods used are wax printing, photolithography, inkjet printing and dip coating.

2.2.1 Conductive inkjet printing

Inkjet printing equipment is the technique used without the need for additional instrumentation. It is widely used because of its reduced cross contamination between samples and reagents and non-contact operation. Canon inkjet printer and ink cartridges are commercially available modified printers that are being

used to print biomolecules for sensors. However, either a modified inkjet printer or an expensive bio-ink printer is necessary for this fabrication technique [29].

2.2.2 Photolithography

Photolithography technique has been used for paper-based sensors with high resolution in large scale production. It involves the use of photoresist, which is poured onto the paper and baked. Further, the paper is irradiated with UV light and that paper should be a patterned transparent film. Then with the help of acetone, un-polymerized photoresist is removed and dried. To create hydrophilic area this paper is exposed to air plasma. This procedure involves long and complex steps and requires the use of expensive processing equipment. However, the formed sensors may damage when it is bent or folded [30].

2.2.3 Wax printing

Wax printing technique is simple, non-toxicity, and low cost compared to other technique. Paper-based microfluidics devices have been fabricated using the wax printing technique[31]. This process occurs by controlling the flow of fluid and wax patterns are directly printed on the paper, that includes the samples and the reagents on the paper. This process is not suitable as the wax melts at high temperature.

2.2.4 Dip coating

It is the simplest method for the fabrication of CP, where there is no necessity of other techniques or instruments. In this technique Whatman filter paper is dipped in the solution of PEDOT: PSS doped with nanomaterial for 10-60 min and dried in an oven. These consecutive cycles can be repeated, and the thickness is proportional to the number of dipping on the paper substrate. Pre-treatment on paper using tween 20 or any other surfactant enhances the overall efficiency of coating[32].

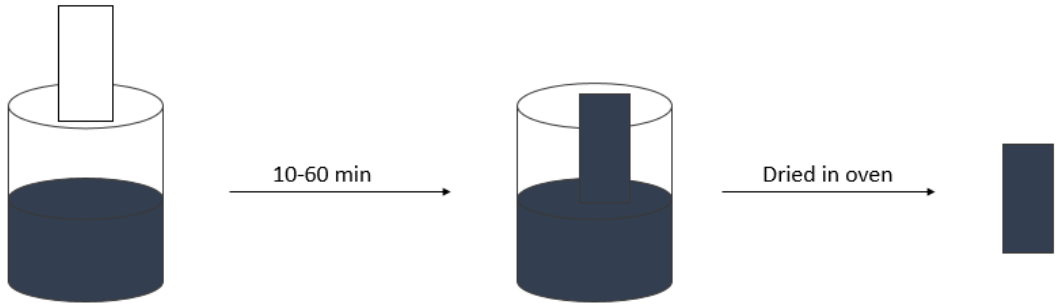

Fig 2.3 Pictorial representation of dip coating method

2.3 Applications of conducting paper in biosensors

CPs are being widely used in the field of biosensors for disease diagnostics, environmental agents, detection of pathogens and for water and food quality maintenance. It can be an alternative to standard laboratory tests and have been widely studied as per their sensitivity and reliability. Compared to the conventional electrode CP based electrochemical biosensors are flexible, cost effective, light-weight and could be easily disposed off[33]. Kumar et al. used composites of PEDOT: PSS and CNT for fabrication of the CP by a simple dip coating method. It was witnessed that the conductivity of the paper increased to two orders of magnitude when it was treated with formic acid. The fabricated CP based electrochemical sensor was found to be flexible, efficient conductive and was disposable. In another work, the CNT was replaced with rGO resulting in improved electrochemical activity and signal stability. Both these CP based sensors were used for the detection of cancer biomarkers, and enhanced sensitivity, lower detection limit, and more extensive linear detection range were observed [32]. The CP based biosensors have been employed for the electrochemical detection of glucose, lactate, and uric acid. For detection of these analytes, corresponding enzymes and electron-transfer mediators are stored in test zones that react with the analytes to produce electrical signals. CP based biosensors are biocompatible and can be conveniently used for cell cultures, to analyze small molecules that are secreted by cells. Since the recognition element attachment and target hybridization can cause agitations in the chain conformation of conducting polymer films,

a binding can be converted to an electrical read-out providing fast, label-free and sensitive measurements. This feature makes conducting polymers excellent candidates as biosensors[20].

Recently, paper based microfluidic approaches enable the fabrication of low cost, simple, flexible, and portable diagnostic platforms. The paper-based microfluidic devices can be easily fabricated using wax printing techniques. Further, these platforms have been used to detect biological analytes (blood, urine, saliva, sweat, tear), environmental reagent (hydrogen sulfide gas, heavy metal ion). Martinez et al. reported the first MF paper-based analytical device (μPAD) for chemical analysis[34]. Nowadays, different techniques are being used for the fabrication of μPAD that is further chemically modified and sealed. These μPAD can be utilized for the detection of various analyte using different transducers. However, further research is needed for the improvement of the μPAD for clinical diagnostics.

Paper based dipstick assays, lateral flow, and vertical flow immunoassays are used for rapid detection of target analytes. Although the dipstick technique is convenient and easy to interpret, the main drawbacks included long analysis times and inaccuracy. Nowadays, lateral flow assays became standard platforms for other applications including screening blood coagulation, detection of pesticides in beverage and food sample and detection of pathogens[35]. Conducting paper based biosensors have also been used for the determination of food safety to ensure the prevention of infection as well as to maintain the necessary nutrients in food. Terzi et al. fabricated a CP based amperometric biosensor for determination of glucose and total carbohydrates in foodstuff[36].

2.4 Importance of conducting polymers in biosensors

Conducting polymers have attained much interest as a suitable matrix for biosensor fabrication. Conducting polymers are utilized to enhance speed, sensitivity and versatility of biosensors in diagnostics to measure vital analytes. That's why the use of conducting polymers are increasing in diagnostic medical reagents [14]. The technique of incorporating enzymes into electro depositable conducting polymeric films

permit the localization of biologically active molecules on electrodes of any size or geometry and is particularly appropriate for the fabrication of multi-analyte electrochemical biosensors. Electrically conducting polymers have noticeable flexibility in the available chemical structure, which can be modified as required. By chemical modeling, it is possible to modulate the required electronic and mechanical properties. Moreover, the polymer itself can be modified to bind protein molecules [37] Significance of conducting polymers are to enhance sensitivity, speed and versatility of biosensors in diagnostics of diseases by measuring vital analyte [19]. Another advantage offered by conducting polymers is that the electrochemical synthesis allows the direct deposition of the polymer on the electrode surface or paper, while simultaneously trapping the molecules [38]. It is thus possible to control the spatial distribution of the immobilized enzymes, the film thickness and modulate the enzyme activity by changing the state of the polymer. The development of any kind of technology in this field heavily depends on the understanding of the interaction at the molecular level, between the biologically active molecule, either as a simple composite or through chemical grafting. For the proper impart of the electrons from the surface of the electrode to the enzyme active site, the concept of 'electrical wiring' has been reported[39]. Conducting polymers are likely to provide a 3-dimensional electrically conducting structure for this purpose.

Conducting polymers are also known to be compatible with biological molecules in neutral aqueous solutions. They can be reversibly doped and undoped electrochemically accompanied by significant changes in conductivity and spectroscopic properties of the films that can be used as a signal for the biochemical reaction. The electronic conductivity of conducting polymers changes over several orders of magnitude in response to changes in pH and redox potential of their environment. Thus conducting polymers have the ability to efficiently transfer electric charge produced by the biochemical reaction to electronic circuit [40].

2.4.1 PEDOT: PSS

Poly (3,4-ethylenedioxythiophene) doped with poly(styrene sulfonate) (PEDOT/PSS) is a widely used conducting polymer for various applications. This is a blend of PSS polyanion with cationic polythiopene derivative. PEDOT: PSS is conducting where the hydrophobic PEDOT-rich chain is encapsulated by hydrophilic and insulating PSS chain. [41]. Kim et al. reported in 2002 that by addition of polar organic solvent, such as dimethyl sulfoxide, N,N-dimethylformamide or tetrahydrofuran into PEDOT: PSS aqueous solution, the conductivity of PEDOT: PSS enhances. It is found that polar organic solvents with high boiling point, such as Ethylene glycol, glycerol, nitro methanol, and other organic solvents with multiple hydroxyl groups, can significantly enhance the conductivity to about 200 Scm^{-1} for PEDOT: PSS prepared from aqueous solution [42].Figure 2.4 shows the structure of PEDOT: PSS.

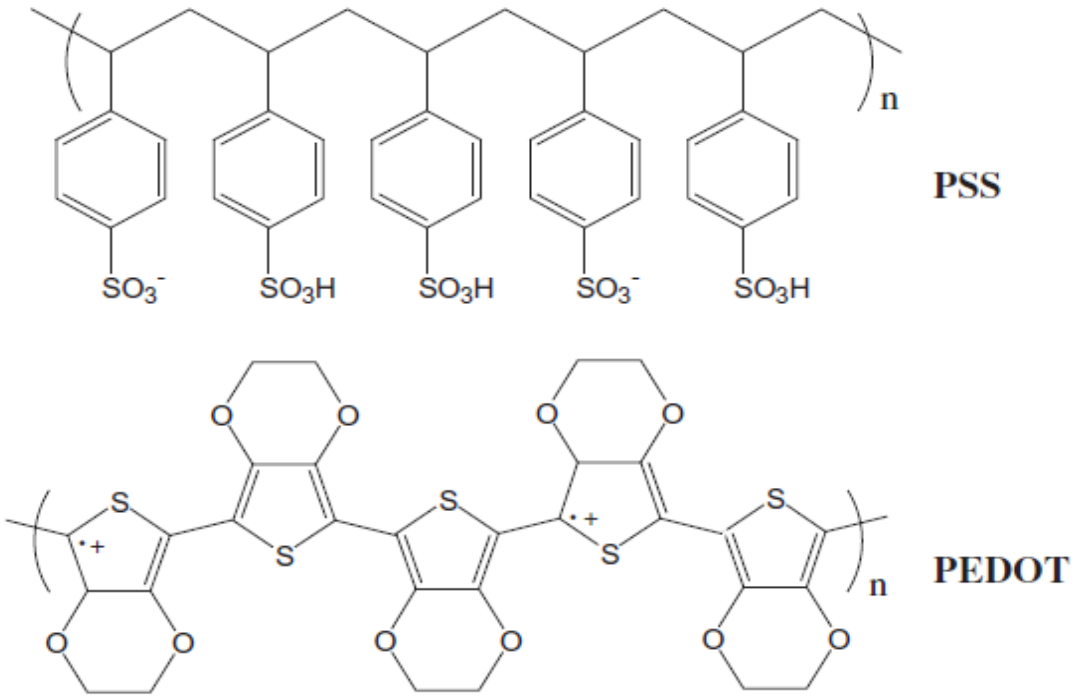

Fig 2.4 Structure of PEDOT: PSS [41]

2.5 Enzyme immobilization

Immobilization of enzyme is an important parameter for the development of high performance biosensor with enhanced sensitivity, high selectivity, excellent operational and storage stability, high reproducibility and short response time. Immobilized biomolecule have to maintain their structure, function, to retain their biological activity after immobilization, to remain tightly bound to the surface and not to be desorbed during the use of biosensor[43]. Ideally, the immobilization process should be simple and effective and also should not cause damage to the activity of corresponding recognition probe. Electrochemical entrapment, physical adsorption, covalent attachment, and affinity interactions are all majorly used methods to immobilize the recognition elements on or within the CPs[17]. So far, different matrices have been used in literature to enhance the performance of the immobilized enzymes. Among these nanomaterials because of their unique physico-chemical properties constitute unique and interesting matrices for enzyme immobilization. The nanomaterials shows characteristics to equilibrate principal factors which determine biocatalysts efficiency, including specific surface area, mass transfer resistance and effective enzyme loading[44].

2.6 Graphene

Graphene is a sp^2 hybridized carbon atoms in hexagonal form in 2D direction. It has remarkable and unique biophysical and chemical property with excellent thermal, electronic, mechanical and optical properties. As they are not reactive, they are functionalised to graphene oxide (GO) forming hydroxyl, carboxyl, ethyl functional groups on the graphene plane, but those are insulated in nature. Reduction of GO to reduced graphene oxide (rGO) is necessary to recover almost the conjugated network and electrical conductivity of pure graphene (figure 2.2). The carboxyl, hydroxyl or epoxy groups bonded on graphene and other atomic-scale lattice defects transform the electronic structure of graphene sheet and serve as strong scattering centers that can affect the electrical transport [45].

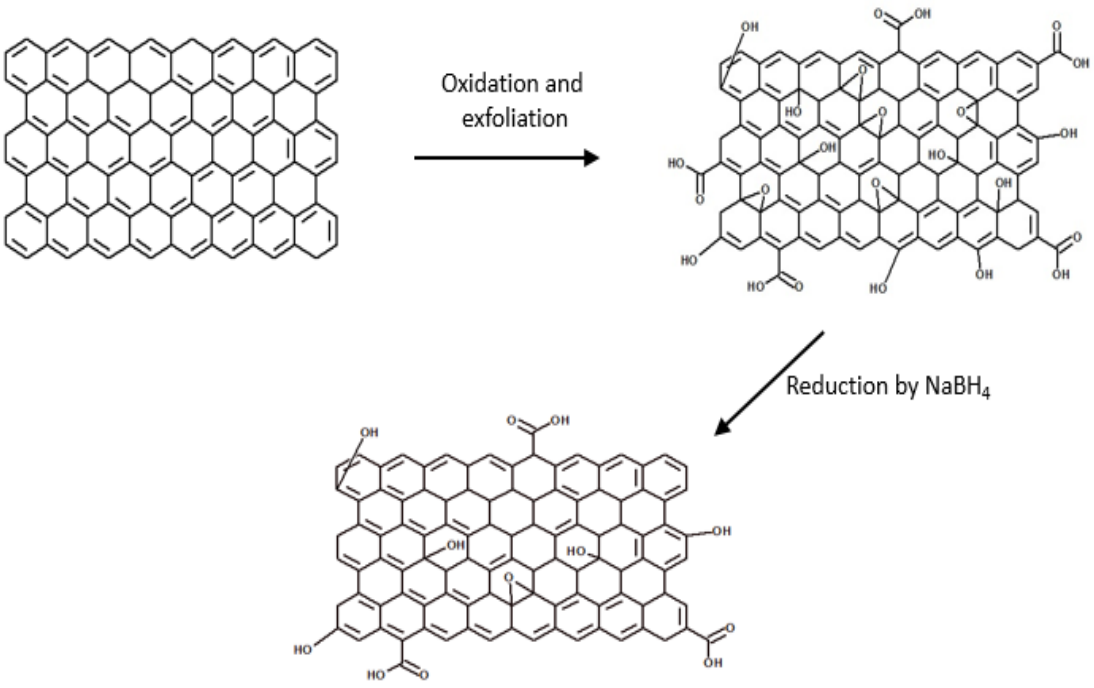

Fig 2.5 Schematic showing the conversion of graphene to reduced graphene oxide

Properties of graphene

- Thinnest material
- sp^2 carbon atom
- Better sensing performance
- Good specific surface area
- Excellent electrical conductivity
- Excellent electron mobility
- Exceptional biocompatibility
- High mechanical strength
- Easy functionalization
- Cost-effective

2.7 TiO_2

TiO_2 is commonly known as the most available and commercially cheapest nanoparticle. Nanostructured TiO_2 is exceptionally versatile and offers unique characteristics such as abundance, chemical stability, long term stability, low cost, biological inertness and electronic properties. Also, their protective effect on harmful UV radiation is much better. However, it is well known that the size and morphology of TiO_2 particles play crucial roles in its properties and applications [46].

Properties of TiO_2 nanoparticle

- Long-term stability
- Biocompatibility
- High surface to volume ratio
- Enhance catalytic activity and selectivity
- Superior thermoelectric property
- Low cost and easily available

2.8 Graphene/TiO_2 nanocomposite

The combination of TiO_2 with graphene could reduce the level of electron-hole recombination, extend electrical conductivity and elevate adsorption capacity thereby enhancing the electrochemical behavior of TiO_2. Previous studies that TiO_2 combined with reduced graphene oxide (rGO) derived via oxidation of graphene and by reduction (rGO/TiO_2 composites) had superior performance to unmodified TiO_2 [47].

3. MATERIALS AND METHODS

3.1 Materials required

PEDOT: PSS (2.8 wt%), TiO_2 nanoparticle, Graphite, glucose oxidase, were procured from Sigma Aldrich, India. H_2SO_4, $NaNO_3$, $CaCl_2$, $KMnO_4$, HCl, ethylene glycol, $NaBH_4$, Methanol, Ethanol and H_2O_2 were procured from Thermofischer Scientific, India.

3.2 Synthesis of graphene oxide

Modified Hummer's method was used for the synthesis of Graphene oxide. The thermal treatment of solution prepared, results in both oxidation and exfoliation of graphite sheets. 2g graphite powder and 2g $NaNO_3$ for the synthesis were added in 90 mL of H_2SO_4 in a 1000 mL volumetric flask. The flask is kept on a magnetic stirrer by placing it in an ice bath (0-5ºC) to control exothermic reaction and continuously stirred for almost 4h at this temperature. Then 12g of potassium permanganate was slowly added to the suspension by maintaining the reaction temperature lower than 12º C. 184 mL of water was slowly added to dilute the mixture and stirred for 2 h. The ice bath was then removed, and the stirring of the mixture was continued for 2 h at 35°C. The obtained mixture was refluxed for 98°C for 10-15 min. After 10-15 min, the temperature was reduced to 30°C resulting in the change of the color of the solution to brown. 40 mL H_2O_2 is added to above solution that resulted in the change of color of the solution to bright yellow. This solution is then mixed with 200 mL of water taken in a beaker and stirred for almost 1 h for proper dilution. This solution was centrifuged using water and HCl for several times. The precipitate was collected and dried at 60°C [48].

3.3 Reduction of graphene oxide

Synthesised GO powder was diluted in DW (0.3 mg/mL). Then 2.28g $NaBH_4$ (reducing) agent and 0.5g $CaCl_2$ (catalyst) was added to a 200 mL GO suspension. The mixture was stirred for 10h at room temperature to obtain reduced graphene

oxide (rGO). The rGO solution formed was filtered and washed by DW until neutralization and dried in an over at 80°C.

3.4 Fabrication of PEDOT: PSS based CP

For the fabrication of conducting paper, whatman filter paper was cut into 1cm × 3cm dimension. This paper electrode were further dipped in an ethanol solution and ultrasonicated for 10 min, followed by rinsing it with distilled water and dried in an oven at 60°C. This paper was dipped in aqueous suspension of PEDOT: PSS (2.8wt%, 5% EG) for 1h and was then dried at 60°C in a oven

3.5 Fabrication of PEDOT: PSS/TiO_2 based CP

The PEDOT: PSS/TiO_2 composite was prepared by doping TiO_2 nanoparticle with PEDOT: PSS (2.8wt%, 5% EG) solution. For this, 50 mg of TiO_2 in DW was ultrasonicated for 30 min and then PEDOT: PSS was added to the dispersion and ultrasonicated for 15 min. Then the above paper strips were dipped into the solution of PEDOT: PSS/TiO_2 for 1h and then dried in an oven. This dipping and drying step was repeated until required thickness with desirable mechanical strength for paper was obtained. This number of dipping corresponds to the number of layer of PEDOT: PSS/TiO_2 deposited.

3.6 Fabrication of PEDOT: PSS/rGO based CP

The PEDOT: PSS/rGO composite was prepared by doping rGO suspension with PEDOT: PSS (2.8wt%, 5%EG) solution. 20 mg of rGO in 40mL of ethanol was ultrasonicated for 60 min and then added to PEDOT: PSS (2.8wt%, 5% EG) solution followed by 60 min sonication. The paper strips were then dipped into the solution of PEDOT: PSS/rGO for 1h and then dried in an oven. This dipping and drying step was repeated until required thickness with desirable mechanical strength for paper was obtained.

3.7 Fabrication of PEDOT: PSS/rGO/TiO_2 based CP

The rGO/TiO_2 nanocomposite was doped with PEDOT:PSS (2.8wt%, 5% EG) aqueous solution to fabricate conductive paper. 20 mg of rGO in 50mL of ethanol was ultrasonicated for 35 min after that 50 mg of TiO_2 nanoparticle was added into the solution and it was sonicated for 2h. Thereafter, this dispersion was doped with 10mL PEDOT: PSS aqueous solution followed by 30min sonication and 3h continuous stirring. The paper strips were then dipped into the solution of PEDOT: PSS/rGO/TiO_2 for 1h and then dried in an oven.

3.8 Fabrication of PEDOT: PSS/rGO/TiO_2 based biosensor

The fabrication of this bioelectrode 20µL of GOx solution was immobilized onto the conducting paper. Thus physically GOx solution is adsorbed onto the paper electrode.

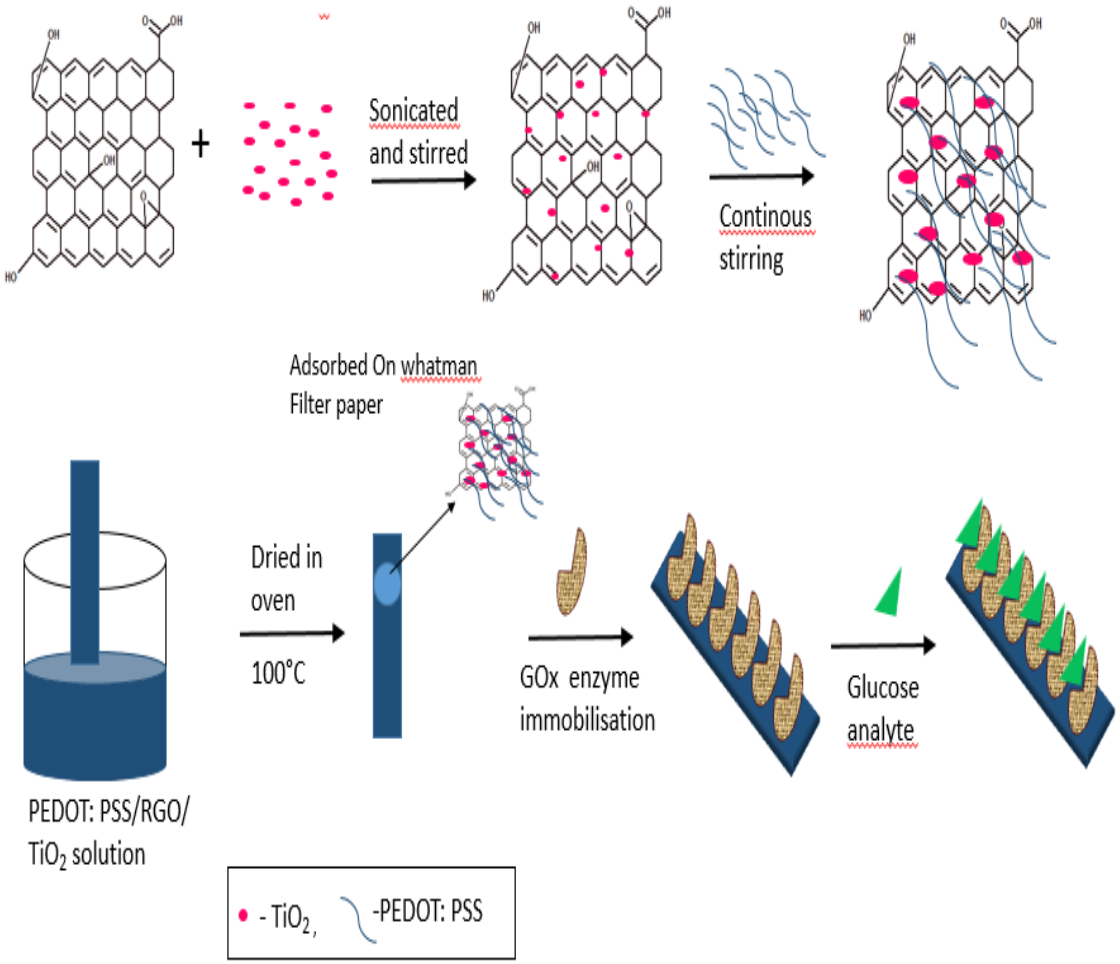

Fig 3.1 Scheme showing the fabrication of rGO/TiO_2 modified conducting paper based biosensor

3.9 Characterization

The conductivity of the CP were calculated using four probe techniques with digital microvoltmeter (model: DMV 001) and low current source (model: LCS 02). The surface morphology of the CP electrode and paper sensor was analyzed by high resolution field emission scanning electron microscopy (model: JSM- 7800F). FTIR of GO and rGO was carried out using Nicolet^{TM} iS10 FTIR spectrometer. X-ray diffraction studies were performed using Brukers XRD D8 discover instrument. Differential scanning calorimetry was performed using DSC 8000, PerkinElmer. Thermogravimetric analysis was performed using TGA 4000, PerkinElmer. Electrochemical studies of paper sensor were carried out by galvanostat/potentiostat (Autolab, Netherland) using the conventional three-electrode cell with the platinum as auxiliary electrode and Ag/AgCl as reference electrode and electroactive paper as working electrode, in phosphate buffer saline (PBS, 100 mM, pH 7.4) containing 5 mM $[Fe(CN)6]^{3-/4-}$.

4. RESULTS AND DISCUSSION

4.1 Conductivity studies

Conductivity studies have been carried out using four probe techniques. The pristine PEDOT: PSS coated paper shows conductivity of 6.9 x 10^{-5} S/cm. However on addition of 5% ethylene glycol in PEDOT: PSS solution, conductivity increases to 1.1 x 10^{-4} S/cm. This is because of the decreased columbic interactions by induced screening effect between PEDOT and PSS molecules, which probably encourage the reorientation of the polymer chains resulting in enhanced charge carrier mobility. EG can provide better flexibility to CP which enriches the property of paper based sensor. As EG is a polar solvent with better dielectric strength, it can increase its conductivity by uncoiling the polymer chain by ejecting PSS chain. Further the addition of TiO_2 nanoparticle into the solution, the conductivity was measured to be 4.1 x 10^{-4} S/cm, which is not a noticeable improvement in conductivity due to the recombination of electron and hole pair of TiO_2 which hinders its electron mobility. At the same time, incorporation of rGO into the PEDOT: PSS matrix shows increase in conductivity by almost 200 S/cm (2.6 x 10^{-2} S/cm). On the other hand modified CP with rGO/TiO_2 nanocomposite showed increased conductivity of 4.9 x 10^{-2} S/cm. This is increased by reducing the recombination of hole and electron pair characteristics of TiO_2 nanoparticle by rGO and by increasing surface area of rGO by TiO_2 nanoparticle. Thus rGO and TiO_2 nanoparticle helps synergetic effect resulting in increase in conductivity.

The electroactive paper thus prepared shows better degree of flexibility in both directions (-180° to +180°) with effectual conductivity. Repeated folding and unfolding of paper didn't affect conductivity of the electroactive paper much, even though tearing off of cellulose thread of paper leads to small variation in conductivity after continuous folding of paper (figure 4.1). Table 4.1 shows the conductivity measurement of different fabricated electrode.

Table 4.1 Conductivity measurement of different fabricated electrode

Conducting paper	**Voltage (volts)**	**Current (ampere)**	**Resistivity (ohm.cm)**	**Conductivity (S/cm)**
PEDOT:PSS	20.29	1.4×10^{-3}	14492.754	6.9×10^{-5}
PEDOT:PSS + EG	2.75	1.4×10^{-3}	8938.923	1.1×10^{-4}
PEDOT:PSS + EG + TiO_2	0.819	1.4×10^{-3}	2401.176	4.1×10^{-4}
PEDOT:PSS + EG + rGO	7.81×10^{-3}	1.4×10^{-3}	38.079	2.6×10^{-2}
PEDOT:PSS + EG + rGO + TiO_2	6.29×10^{-3}	1.4×10^{-3}	20.43	4.9×10^{-2}

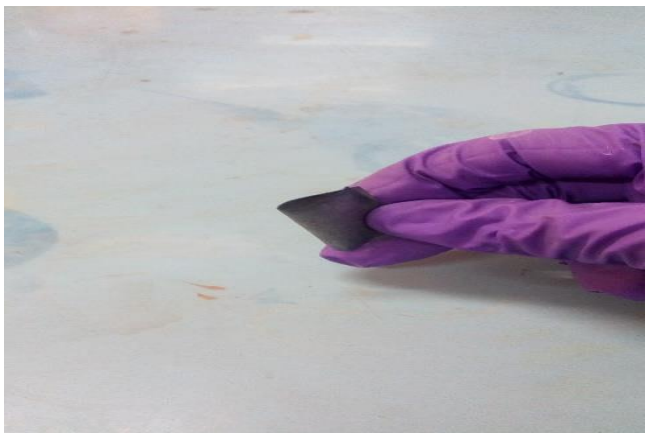

Fig 4.1 Image showing foldable nature of electro-active paper.

4.2 SEM Analysis

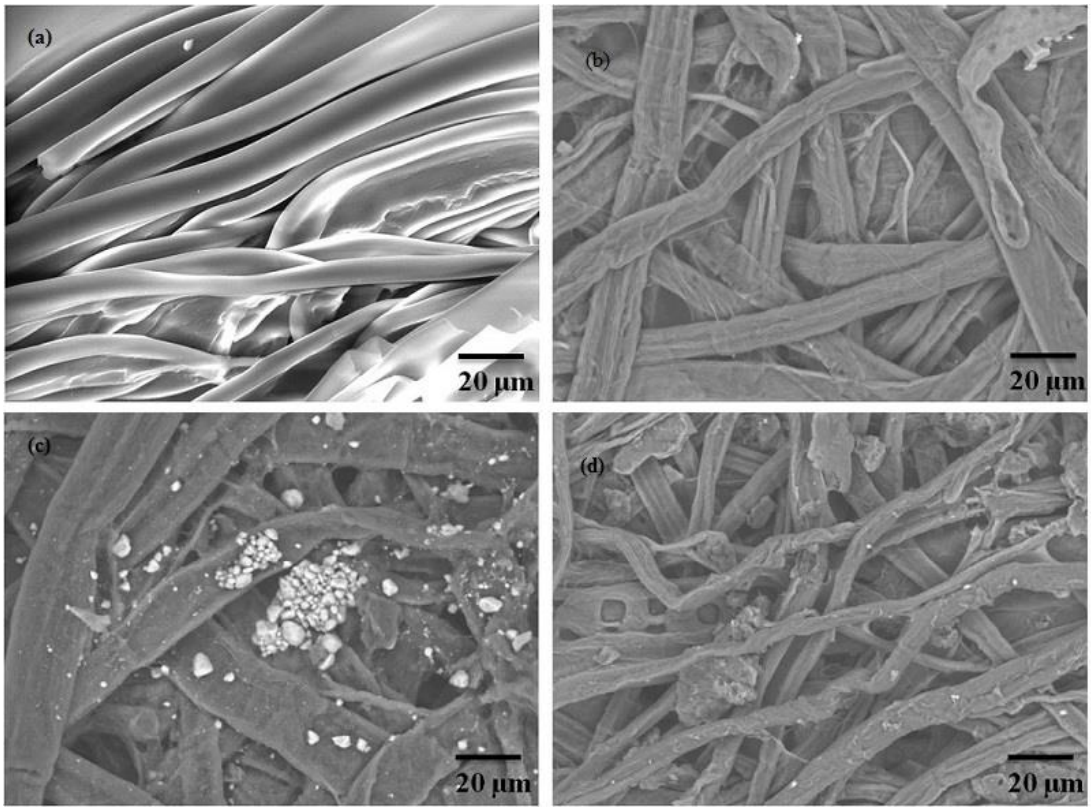

Fig 4.2 SEM images of (a) Paper electrode, (b) PEDOT: PSS doped with EG, (c) PEDOT: PSS/EG doped with TiO_2, (d) PEDOT: PSS doped with rGO/TiO_2

The surface morphology of paper was analyzed by SEM. Fig 4.2(a) indicates the SEM image of paper electrode . Fig 4.2 (b) shows SEM image of PEDOT: PSS doped with EG coated filter paper. It shows uniform distribution of PEDOT: PSS over the cellulose fiber of the Whatman filter paper. Because of the high porosity and inter-fiber tiny air spaces in Whatman paper, the PEDOT: PSS adsorbs into the paper by capillary action and after drying it produces uniform and stable film. Fig 4.2(c) shows the SEM image of PEDOT: PSS/ EG doped with TiO_2. It shows that the TiO_2 nanoparticle has adsorbed on the CP. Fig 4.2(d) shows the SEM image of PEDOT: PSS modified with rGO/TiO_2. Here rGO sheets and TiO_2 nanoparticles are uniformly entangled in the fibrous network of Whatman filter paper.

4.3 XRD Analysis

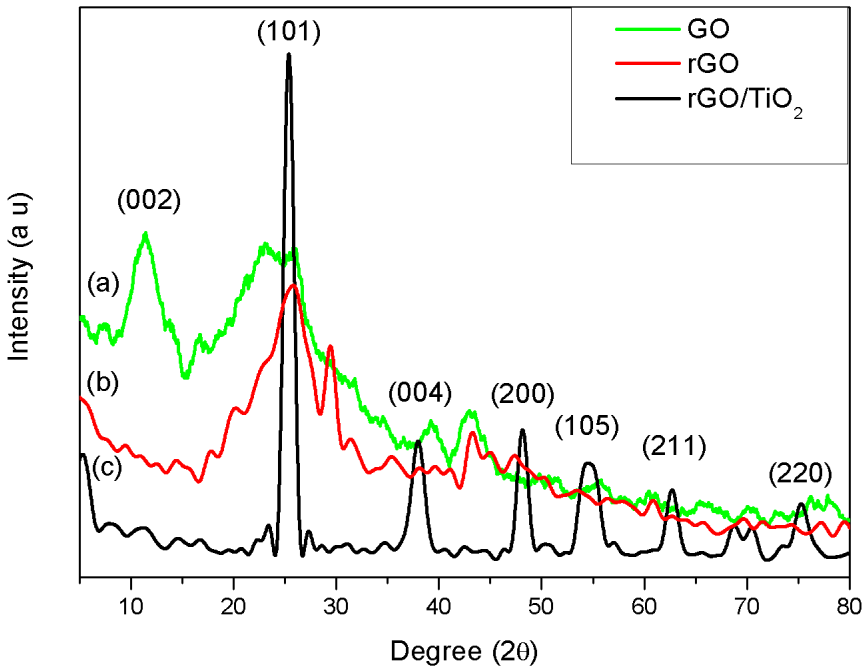

Fig 4.3 XRD spectra of (a) GO, (b) rGO, (c) rGO/TiO_2 nanocomposite

The XRD of GO shows peak at 11° (corresponding to 002) lattice plane corresponding to d spacing value of 0.81 nm was observed for the GO powders as a result of the presence of oxygen-containing groups between GO sheets. Also, there is a broad peak, with weaker intensity seemed in the range of 20–26° with corresponding d-spacing of 0.37 nm –0.52 nm. In the case of rGO, the peak at 11° has disappeared by the reduction and removal of oxygen containing group by $NaBH_4$ and so dismantling of regular stacking of GO sheets in GO has also vanished, thus aggregation of graphene was much reduced in rGO. The broad peak in 20–26° has retained, indicating parallel stacking of the rGO sheets. In the case of RGO-TiO_2 characteristic peaks at 25.1°, 37.4°, 47.6°, 55.3°, 62.9° and 75.2° corresponded to the (101), (004), (200), (105), (211) and (220) crystal planes of anatase TiO_2, with lattice constants of $a = b = 3.7862$ ˚A and $c = 9.5209$ ˚A. It was observed that all the peaks indicates anataseTiO_2 phase and show no particular peaks of GO or rGO layered structure, that might be because of the relatively low diffraction intensity of rGO that was detained by the (101) crystal peak of TiO_2.

4.4 FT-IR of GO and rGO

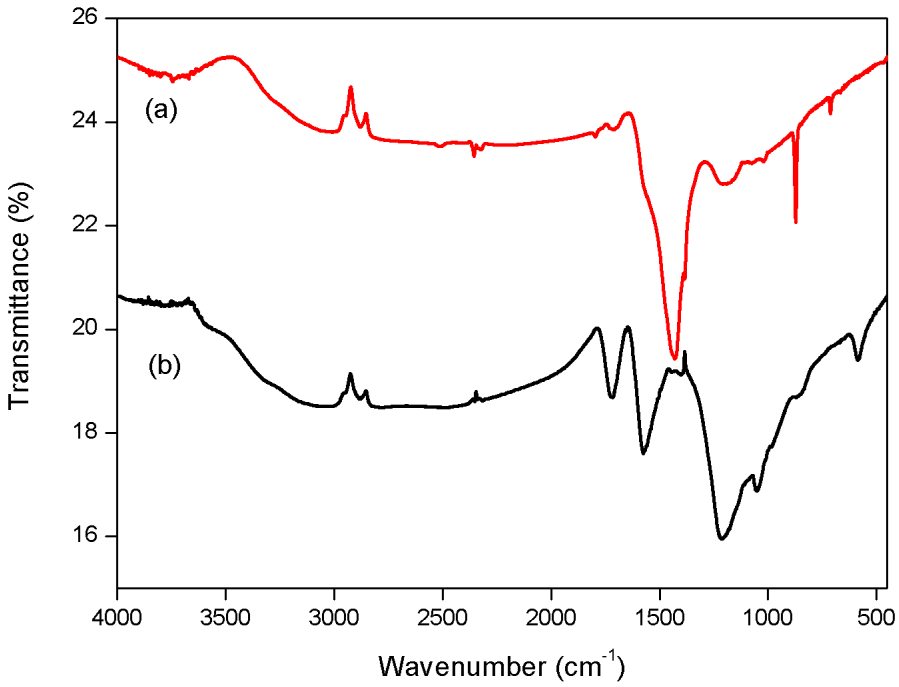

Fig 4.4 FT-IR spectra of (a) rGO and (b) GO.

FT-IR spectra of GO indicates characteristics absorption broad band at 3013 cm^{-1} that corresponds to the stretching vibration of hydroxyl group (OH). The band at 1715 cm^{-1} represents the C=O stretching vibration in the COOH group of GO. The band seen at 1574 cm^{-1} is due to the C-C stretching of the aromatic in the graphene. Then the presence of ether group (C-O-C bond) is shown by the absorption band at 1211 cm^{-1}.

FT-IR spectra of rGO also shows OH peak near 3009 cm^{-1}but the intensity of peak was reduced. Further the band at 1715 cm^{-1}in GO is removed, which indicates that C=O stretching is eliminated that means the carboxyl group is reduced properly. CH_2 vibration is induced in the RGO at 1430 cm^{-1} because of the reduction of carboxyl and ether group. Sharp peak at 873 cm^{-1} is due to the C-CH_2 stretching vibration.

4.5 FT-IR of electroactive paper

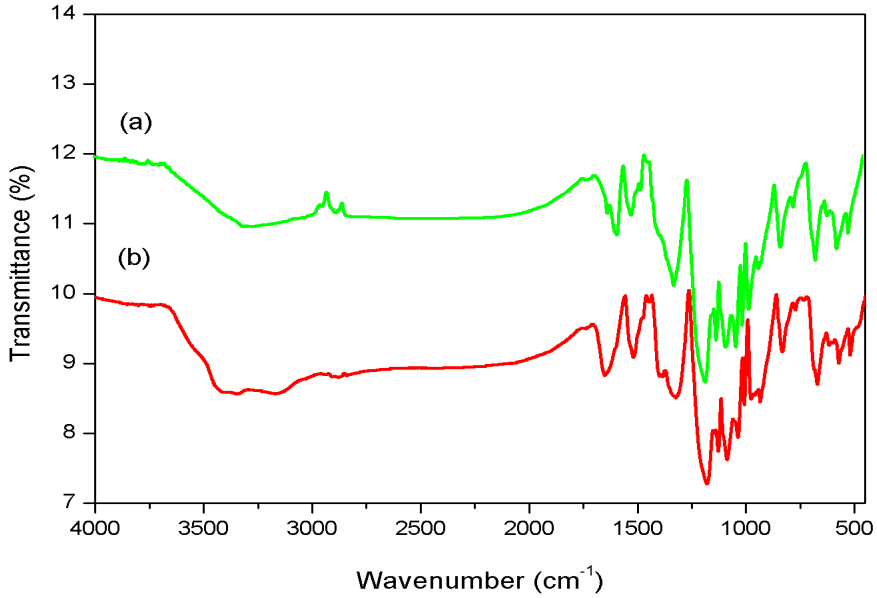

Fig 4.5 FT-IR spectra of conducting papers (a) PEDOT: PSS (2.8wt%, 5% EG) with RGO (b) PEDOT: PSS (2.8wt%, 5% EG) with RGO/TiO_2

FT-IR spectra of electroactive paper of PEDOT: PSS (2.8wt%, 5% EG) exhibits characteristic absorption bands at 671 cm^{-1}, which corresponding to C–S stretching vibration of the thiophene ring in PEDOT. The band at 832 cm^{-1} and 970 cm^{-1} represents aromatic CH out of plane and in plane bending vibration respectively. The bands at 1007 cm^{-1} and 1037 cm^{-1} indicates SO_3^- symmetric and asymmetric stretching vibrations respectively of PSS molecule. The band at 1324 cm^{-1} is because of the C-O stretching vibration in RGO and the bands shown at 1520 cm^{-1} and 1586 cm^{-1} are because of C-C and C=C vibrations respectively. In the spectra of CP, the absorption band at 1085 cm^{-1} is due to addition of EG that causes conformational changes in the polymer structure whereas disappearance of SO_3^- symmetric stretching proposes molecular rearrangement in the PEDOT:PSS leading to increased electrical conductivity. The absorption band at 1128 cm^{-1} and 1179 cm^{-1} are as a result of the S=O and C-O stretching eliphatic ether respectively in PEDOT: PSS. The broad band at 3300 cm^{-1} represents the presence of OH group. Addition of rGO and TiO_2 didn't make any structural difference in PEDOT: PSS. Incorporation of TiO_2 shifts the conjugated C=C bond to non-conjugated C=C bond.

4.6 Thermal Analysis

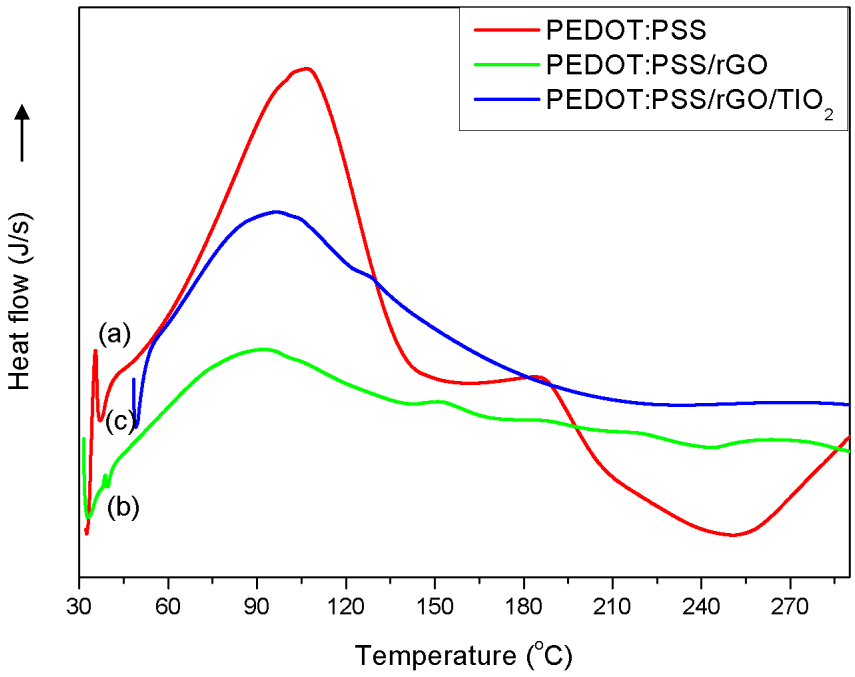

Fig 4.6 DSC plot of (a) PEDOT: PSS, (b) PEDOT:PSS/rGO, (c) PEDOT:PSS/rGO/TiO_2

The DSC curves of PEDOT: PSS/EG films did not show a well-defined glass transition temperature. An exothermic peak was observed around 112°C, indicating the crystallization temperature (Tc). An indication that the sample was free of water is revealed by the absence of a peak near to 0°C. The PEDOT: PSS chains tend to form crystals during film formation or during slow cooling if there is enough time to reorganize themselves. Thereafter a broad endothermic peak is shown around 250°C mentioning its melting temperature (Tm). By the incorporation of rGO and TiO_2, the exothermic peak mentioning crystallization retain but with lesser amount of heat was required, showing its more tendency to form crystalline than pristine PEDOT: PSS. While the temperature increases, it didn't show the endothermic peak mentioning melting point, which means the melting point increases by the incorporation of rGO and TiO_2 because of its structural property. Even though Tg is not specified in those 3 DSC curve.

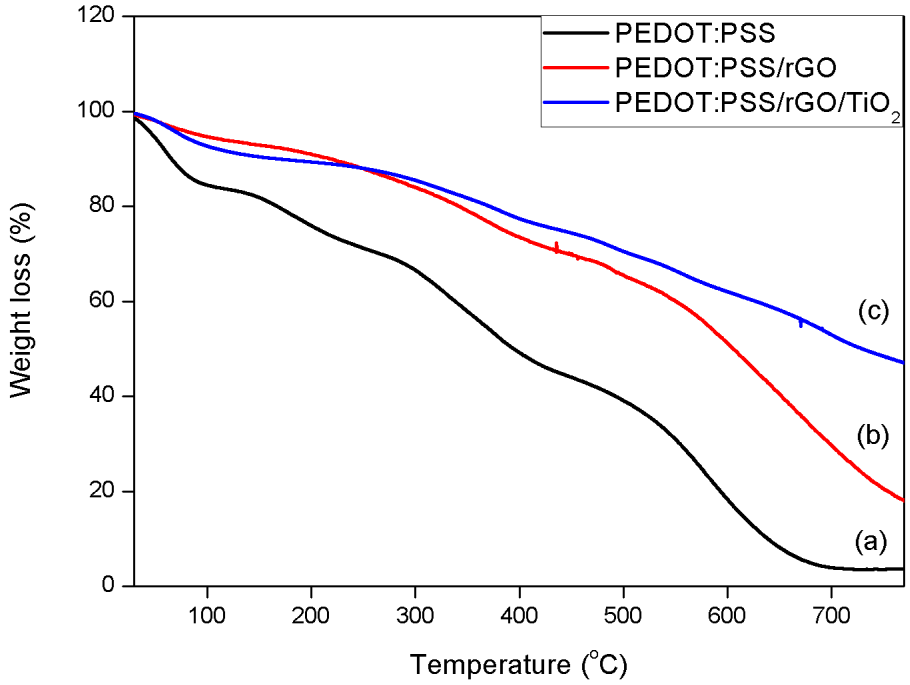

Fig 4.7 TGA plot of (a) PEDOT:PSS, (b) PEDOT:PSS/rGO, (c) PEDOT:PSS/rGO/TiO_2

TGA curve shows the weight loss of PEDOT: PSS, PEDOT: PSS with rGO and PEDOT: PSS with rGO and TiO_2 with respect to temperature. The weight loss at 100°C might be because of the evaporation of moisture content in the films. In the case of PEDOT: PSS, decomposition occurs gradually from 100°C. This step by step degradation occur by the fragmentation of PSS sulfonate group from the polymer chain. At higher temperatures (500°C), other fragments due to carbon oxidation of both PEDOT: PSS and EG are detected and weight loss will be up to 2%. Even by the incorporation of rGO, the PSS fragmentation will be there but in minimized manner because of the rGO stability to thermal effect and the overall weight loss will be up to 20%. In the case of rGO/TiO_2 nanocomposite this gradual weight reduction occurs till 700 °C and the weight loss at that point will only be up to almost 50%. Thus by the addition of nanocomposite, the thermal stability enhanced.

4.7 Electrochemical studies of fabricated electrode

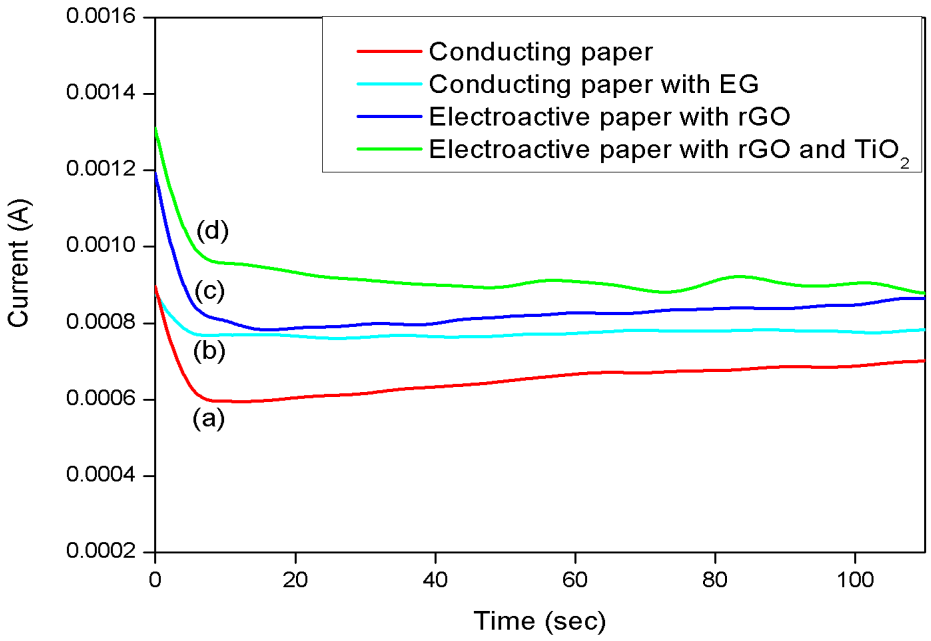

Fig 4.8 Chronoamperometric plot obtained for (a) conducting paper, (b) conducting paper with EG, (c) electroactive paper with rGO, (d) electroactive paper with rGO and TiO_2

Chronoamperometry studies are desirable for estimation of mass change per mole of electron transfer at electrode- electrolyte interface within short period of time at constant potential (2V). Low detection potential is given to diminish the influence of those easily oxidisable species. This plot indicates the current intake on different paper electrode and higher the current value shows higher the electron transfer between solution and different electrodes. In the case of conducting paper doped with EG, the conductivity increases because of conformational rearrangement in the structure of PEDOT: PSS chain. Further rise in the electrochemical current occur by the doping of rGO leads to enhanced penetrability of Ferro-Ferri cyanide into the substrate, which permits the enhancement of sensitivity. With the incorporation of TiO_2, value of electrochemical current slightly increased further, because of its nanostructure having better surface to volume ratio, which can sense more than rGO alone having better conductivity. Thus from this graph, it is revealed that electroactive paper doped with both rGO and TiO_2 shows better electron transfer, which means that, it indulge better sensitivity than others.

4.8 Electrochemical studies of glucose sensing

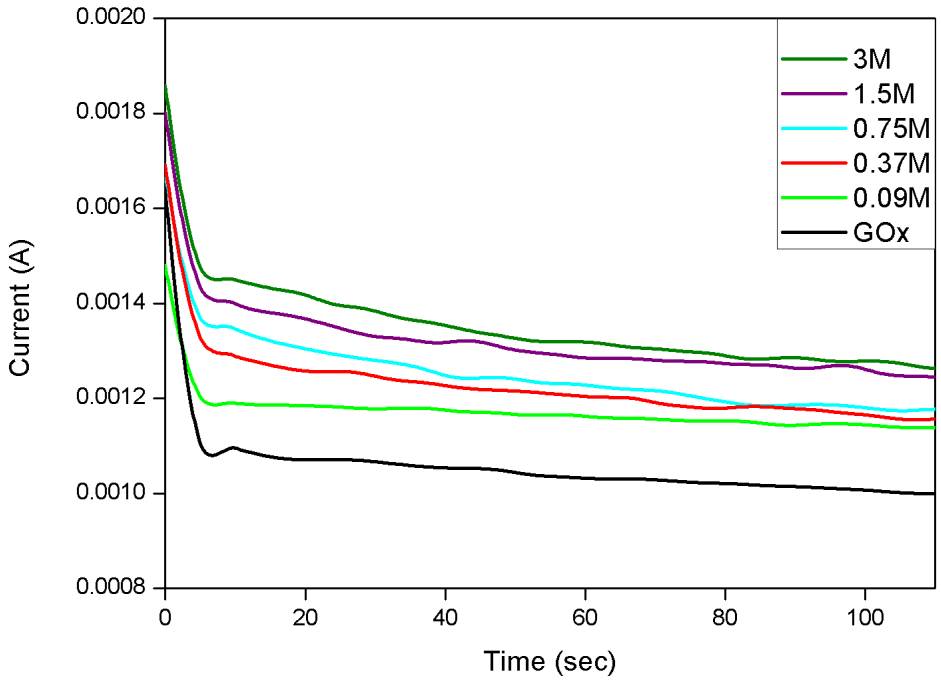

Fig 4.9 Electrochemical response studies of GOx immobilized electro-active paper with different concentration of glucose.

Considering the fact that electro-active paper doped with rGO and TiO_2 have better conductivity, they have been used for the sensing of glucose. For sensing, GOx enzyme diluted in phosphate buffer solution (PBS 3.2mg, pH- 7.4) is immobilized onto the electroactive paper by physical adsorption. Glucose solution with different concentration ie, from 0.09 M to 3M was prepared from the stock solution. The response studies of GOx immobilized CP obtained as a function of glucose concentration. The response current increased from 0.00148-.00184 A with respect to glucose concentration. The sensors shows a linear concentration range (0.09-3M) with a correlation coefficient of 0.99.

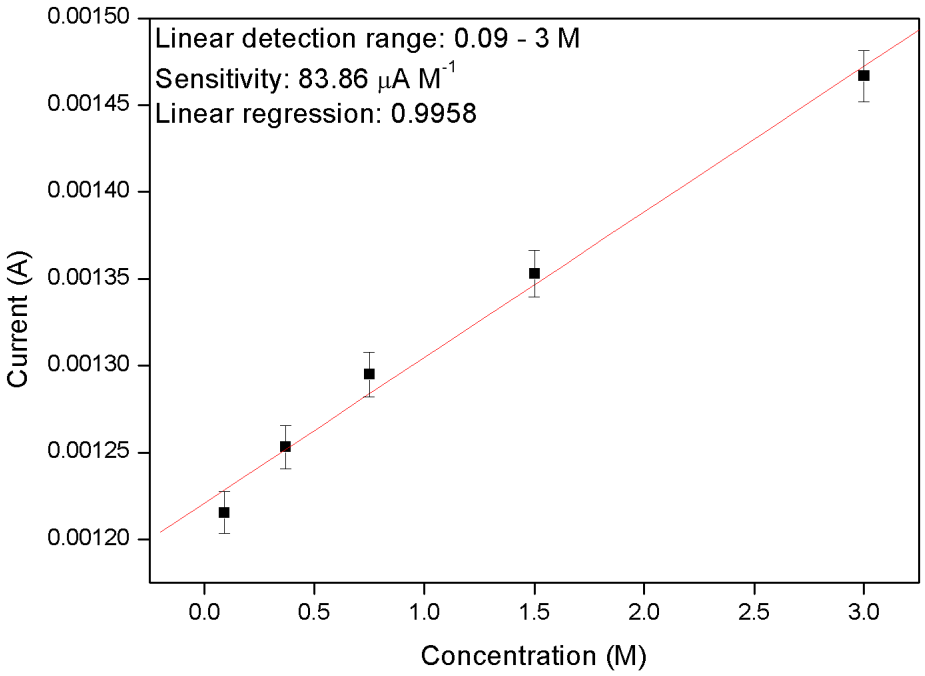

Fig 4.10 Calibration plot between response current and concentration of glucose

Fig 4.10 shows the calibration curve between glucose concentration (M) and response current (A). It is shown that amperometry current increases with glucose concentration. Sensitivity of the biosensor is the slope of the linear line and some point of error is noted. A linear relationship is observed in the range 0.09-3M with the sensitivity of 83.86 μA M^{-1} and follows equation:

I(A)= 0.0021 A + 83.86 μA M^{-1} x [glucose concentration]

R = 0.9958

4.9 Interference study

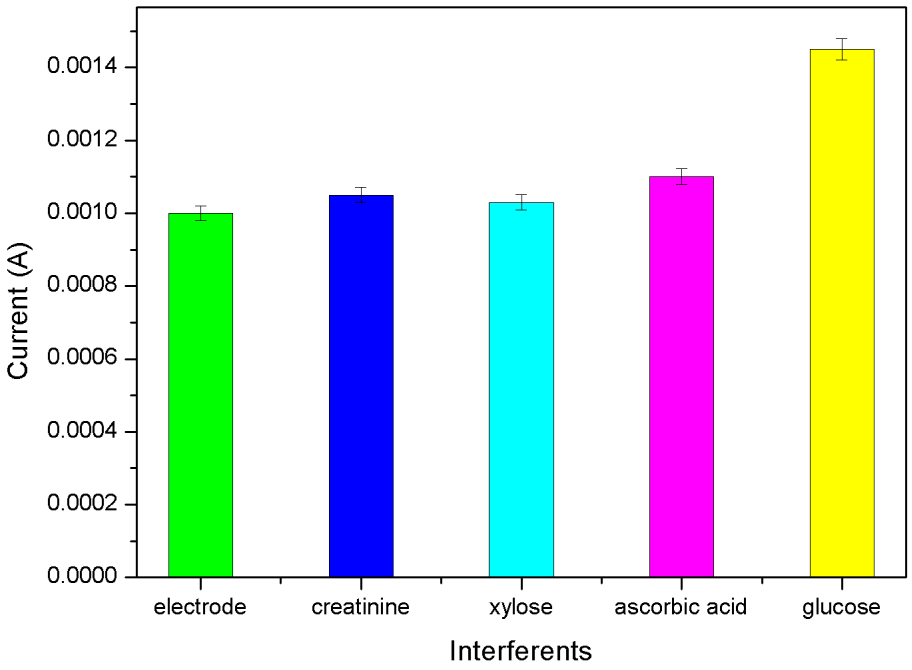

Fig 4.11 Electrochemical current response of paper electrode in the presence of other analytes

Fig 4.11 shows the interferents study of paper sensor that have been conducted by using three different analytes like ascorbic acid, creatinine, xylose. All these analytes have been diluted into solution as same as that of glucose. The result clearly shows that the change in chronoamperometric current response indicating that the modified CP based electrode is highly selective for glucose determination.

5. CONCLUSION

Conducting paper based biosensor has been fabricated using PEDOT:PSS/rGO/TiO_2 nanocomposite. The fabricated electrode shows amended conductivity of $4.9x10^{-2}$S/cm that is 200 S/cm higher than the conductivity of PEDOT: PSS modified paper. This highly flexible, environment friendly and low cost, CP has been used for glucose detection using GOx enzyme. The pretreatment of the CP effectively absorbs GOx without the requirement of any crosslinking agent. The fabricated biosensor shows good sensitivity (83.86 μA M^{-1}) and selectivity towards glucose. The higher sensitivity of the fabricated sensor is due to strong affinity of rGO and GOx introduced due to repeated pre-treatment. Efforts are being made to regulate the concentration of glucose in human serum samples.

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